APPENDIX B: MARKED UP COPY OF AMENDED SPECIFICATION

Page 1, lines 4-8:

The present application is a continuation-in-part of co-pending U.S. Provisional Patent Application Serial No. 60/029,044 filed October 29, 1996. The entire text of the above-referenced disclosure is specifically incorporated by reference herein without disclaimer. The present application is also a continuation of U.S. Serial No. 09/303,161, filed April 29, 1999, and PCT/US97/20170, filed October 29, 1997. The government may own rights in the present invention pursuant to grant numbers RO1 AI30581 and POI CA18221 from the National Institutes of Health.

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Labeling, immunoprecipitation and detection of MICA. For surface labeling, washed cells in phosphate-buffered saline (PBS) were biotinylated with [Sulfo-NHS-LC-biotin] SULFO-NHS-LC-BIOTION™ (Pierce Chemical Co., Rockford, IL) (100 μg/ml) for 30 min at 4° C and reactions quenched by addition of 25 mM lysine. 1-3 x 10^7 cells were lysed in 1 ml lysis buffer [1% Triton X-100, 50 mM Tris-OH (pH 7.4), 150 mM NaCl, 5 mM EDTA, 5 mM iodoacetamide, protease inhibitors]. Protein in cleared supernatants was quantitated with a [MicroBCA] MICROBCA™ kit (Pierce, Chemical Co., Rockford, IL) and lysates were precleared using ULTRALINK-Protein A/G beads (Pierce Chemical Co.). MICA was precipitated with purified mAB 56 and protein A/G beads and immunocomplexes washed. Aliquots were treated with *N*-glycanase (PNGase F, New England Biolabs Inc., Beverly, MA) as recommended by the manufacturer. Dissociated and dithiothreitol-reduced immunocomplexes were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto nitrocellulose using a Trans-Blot Semi-Dry Transfer Cell (Bio-Rad, Laboratories, Inc., Hercules, CA). After overnight incubation of membranes in PBS containing 10% dry nonfat milk, 0.05% TWEEN 20 and 0.02% Na-azide, they were repeatedly washed in TST [0.15 M NaCl, 10 mM Tris-OH (pH 7.4), 0.3% TWEEN 20] and reacted with avidin-horseradish peroxidase

(Vector Laboratories Inc., Burlingame, CA) in TST (2.5 μg/ml) for 1 hour at 4° C. Membranes washed with TST were treated with ECL enhanced chemiluminescent reagent (Amersham, Life Science, Arlington Heights, IL) and exposed to X-ray film. For pulse-labeling and chase, 5 x 10^6 cells per time point were labeled with 0.5 mCi [^{35}S]methionine for 5 min. as described (Grandea *et al.,* 1995). For chase, cells were spun through PBS with 10 mM methionine and resuspended in growth media for the indicated time periods. Cells were lysed, and MICA protein was precipitated using mAB 2C10 as described above. Isolated and denatured MICA was treated with endoglycosidase H (Endo H, New England Biolabs) as recommended by the manufacturer and analysed by SDS-PAGE. Fixed gels were treated with AMPLIFY (Amersham) and dried for autoradiography.